Effect of apple pectin on gut microbiota - qPCR in applied microbiology

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Effects of apples and specific apple components on the intestinal environment of conventional rats: Role of apple pectin

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Introduction: This study was part of the ISAFRUIT project, which aims to reveal biological explanations of the health effects of fruits. A number of health related targets may be affected by the intestinal microbiota e.g. immune system regulation, cancer prevention, enteral infection resistance and obesity control. Generally, the Gram-positive, fermenting bacterial populations (including Bifidobacteria and Lactobacillus) are considered biomarkers of a well-balanced intestinal microbiota, whereas overgrowth of certain *Bacteroides* species is considered undesirable. Moreover, butyrate-producing Clostridial clusters is considered beneficial to the gut mucosa as butyrate functions as a fuel for enterocytes.

The objective of this study was to identify effects of apple and apple product consumption on the microbiotal composition.

Model: Male Fisher rats were subjected to two long-term (14 weeks) and one short-term feeding study (4 weeks) with whole apples or selected apple components. Effects on microbiotal composition was analyzed with Principal Component Analysis (PCA), denaturing gradient gel electrophoresis (DGGE) and quanititative real-time PCR (q-PCR).

Results:

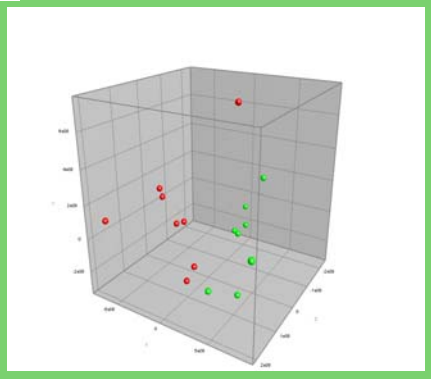

Figure 1: PCA analysis of DGGE profiles from rats fed 14 weeks with 0,33% pectin diet (green) or control diet (red). It is clear that the groups segregate into two separate clusters. Similar findings were seen with higher pectin concentration and with whole apples, but not with other tested apple components.

Hence, a clear difference in microbiotal composition is present, and based on these results the effect is mainly based on the presence of pectin in apples.

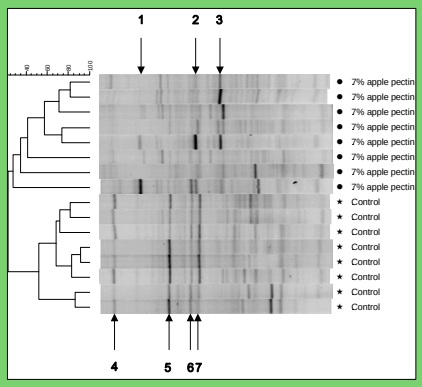

Figure 2: Cluster analysis of universal DGGE profiles from the short-term experiment. Pearson correlation analysis of universal DGGE gel profiles from cecal content of rats fed with either control- or 7% pectin diet for four weeks. Bands were DNA sequenced by GATC (Konstanz, Germany) and arrows represent the Genus: Anaeroplasma (1), Clostridium sp. (2), Clostridiales (3), Bacteroides sp. (4, 6, 7) and Alistipes (5).
2 and 3 belong to the Firmicutes Phylum; 1, 4-7 belong to Bacteroidetes Phylum.

In this short-term experiment, the DGGE profiles showed no clear effect of whole apple consumption as observed in the long-term trial, but as seen here a marked effect of pectin consumption is observed.

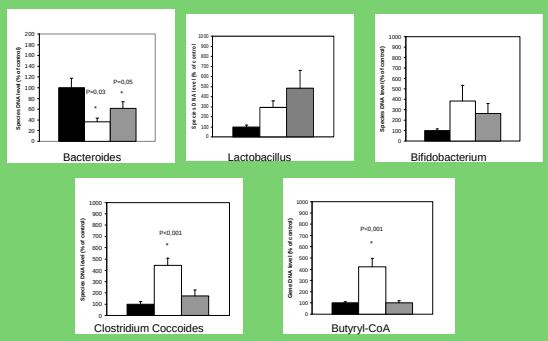

Figure 3: Q-PCR on bacterial composition from the short-term feeding experiment. Relative amount of target gene in samples from animals in the control group (N=8) (black), the pectin-fed group (N=7) (white) and the apple-fed group (N=8) (gray).

DNA amount in the control group was set to 100%. Error bars represent standard errors of the means. The statistical analysis was performed with One-Way ANOVA. Values significantly different from the control are indicated with an asterisk and a p-value.

Note the significant downregulation of Bacteroides and upregulation of Clostridial and Butyrul-CoA levels in the pectin groups in particular.

No statistical significance was found for Lactobacillus and Bifido, but there was a clear trend that the pectin-fed group had higher levels of these bacteria.

Q-PCR – method

Platform
The quantitative changes in bacterial composition were verified by quantitative real-time PCR using SybrGreen on ABI prism 7900HT from Applied Biosystems.

Primer design
Primer sets for 16S rRNA from Lactobacillus, Bifidobacterium and Clostridium coccoides and for the gene Butyrul-coenzyme A transferase were found in the literature, whereas the Bacteroides primer set was designed to amplify a segment of the DNA sequence represented by the highly homologous bands 4-7. ClustalW2 was used to align these 4 sequences and NCBI's primer designing tool was used to design the best primer set.

Data analysis
All results were calculated as ratios of relative expression levels to HDA expression levels in order to correct data for differences in total DNA concentration between individual samples.The HDA primer set amplifies the V2-V3 region of the 16S ribosomal DNA gene, a well conserved bacterial marker region.
DNA levels were approximated as 2^{-Ct}, where Ct is the threshold cycle calculated by the ABI software as the PCR cycle, where amplifications signal exceeds the selected threshold value, also set by the software. All samples were calculated as means of duplicate determinations.

Discussion+Conclusions

The decrease in certain Bacteroides species and increase in Clostridium coccoidales identified in pectin treated animals by DGGE was successfully verified by Q-PCR. Buturyl-CoA is present in butyrate-producing bacteria such as in certain Clostridial clusters, which fits well with a similar 4-fold increase for these primers sets in pectin treated animals.
We also found (not shown) that pectin decreased pH and increased cecal weight, which would indicate increased bacterial fermentation, coinciding well with the tendency to increased Bifido and Lactobacillus levels.

The collected evidence thus suggests that apples have a health-promoting effect on the rat intestinal microbiota, and that this effect is mainly explained by the presence of pectin in the apples.

However, a human being must eat 3 kg apples a day to reach an intake corresponding to 0.15% pectin.
The data presented here will at a later stage be interpreted in the context of other biological changes recorded during the course of the ISAFRUIT project, which includes also human intervention studies.